09/615 872

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PATENT
Docket No. 310.00340101

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Applicant(s):	Arepally et al.	Group Art Unit:	1641
Patent No.:	6,964,854	Examiner:	James L. Grun
Confirmation No.:	4363		
Issued:	November 15, 2005		

For: COMPOSITIONS AND METHODS USEFUL FOR THE DIAGNOSIS AND TREATMENT OF HEPARIN INDUCED THROMBOCYTOPENIA/THROMBOSIS

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JAN 30 2006

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REQUEST FOR CERTIFICATE OF CORRECTION

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Sir:

A Certificate of Correction is requested to be issued correcting printing errors appearing in the above-identified United States patent. Two copies of the text noting the corrections for the Certificate are enclosed. Since none of the errors listed are due to Applicants' mistake, no fee is necessary for the Certificate. The corrections in the proposed Certificate of Correction do not involve such changes in the patent as would constitute new matter or would require reexamination.

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Deb Schurmann
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UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO.: 6,964,854
DATED: November 15, 2005
INVENTOR(S): Arepally et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

On the face page, (73) Assignee, delete "Science & Technology Corporation" and replace with –Science & Technology Corporation @ UNM–.

On the face page, (73) Assignee, delete "Alburquerque" and replace with –Albuquerque–.

In column 16, line 65, delete "215:403410" and replace with –215:403-410–.

In column 22, line 10, delete "intrastemal" and replace with –intrasternal–.

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JAN 30 2006

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(12) **United States Patent**
Arepally et al.

(10) **Patent No.: US 6,964,854 B1**
(45) **Date of Patent: Nov. 15, 2005**

(54) **COMPOSITIONS AND METHODS USEFUL FOR THE DIAGNOSIS AND TREATMENT OF HEPARIN INDUCED THROMBOCYTOPENIA/THROMBOSIS**

(75) Inventors: **Gowthami M. Arepally**, Albuquerque, NM (US); **Walter Kisiel**, Albuquerque, NM (US); **Keiko Kamei**, Kumamoto (JP); **Shintaro Kamei**, Kumamoto (JP)

Science + Technology Corporation @ UNM,

(73) Assignee: ~~Science & Technology Corporation~~, ~~Albuquerque~~, NM (US)
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(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 51 days.

(21) Appl. No.: **09/615,872**

(22) Filed: **Jul. 13, 2000**

Related U.S. Application Data

(60) Provisional application No. 60/143,536, filed on Jul. 13, 1999.

(51) **Int. Cl.**[7] **C07K 16/18**; C12N 5/20; C12P 21/08; G01N 33/564; G01N 33/577

(52) **U.S. Cl.** **435/7.24**; 435/70.21; 435/452; 435/332; 435/337; 436/506; 530/388.2; 530/388.25

(58) **Field of Search** 435/7.24, 7.6, 435/7.92, 69.1, 70.21, 452, 332, 337; 436/506; 530/387.3, 388.1, 388.2, 388.25

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Primary Examiner—Christopher L. Chin
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(57) **ABSTRACT**

The invention includes compositions, kits and methods comprising a monoclonal antibody which shares key functional properties with the polyclonal antibodies which participate in the pathogenesis of heparin induced thrombocytopenia/thrombosis (HIT/HITT) in a mammal. The monoclonal antibody of the invention preferentially binds with a PF4/heparin complex relative to the binding of the antibody with PF4 or heparin alone. The monoclonal antibody of the invention also binds specifically with PF4 in a complex with other glycosaminoglycans besides heparin, and also activates platelets. The monoclonal antibody of the invention is useful in methods for diagnosing and treating HIT/HITT in a mammal. A humanized version of the monoclonal antibody of the invention is also included, along with a process for humanizing the monoclonal antibody of the invention.

6 Claims, 10 Drawing Sheets

murine antibody onto the human heavy and light chain framework residues. The original technique described by Reichman et. al. involves producing a human variable region with the desired specificity of the murine hypervariable region. Murine hypervariable (HV) primers with sequences complementary to the murine HV region are synthesized with flanking human framework sequences corresponding to the human HV region. These murine HV primers, in addition to nucleotides and DNA polymerase, are added to a plasmid containing the human variable region gene. This reaction results in a humanized murine variable gene (huV). containing human framework regions and murine HV regions. The humanized variable region is next inserted into a plasmid containing a human constant region gene.

This procedure was employed to create humanized heavy and light chains which were then cotransfected into a non-secreting myeloma cell line. Subsequent refinements of this basic procedure have resulted in a process for producing humanized antibodies with affinities and biological properties comparable to that of the parent murine monoclonal antibody (Emery et al., 1995, Strategies for humanizing antibodies, In: Antibody Engineering, 2nd ed., Ed. Borrebaeck CAK) pp 159–183, Oxford University Press, Oxford). This technology can be similary utilized to create functional humanized Fab' fragments in bacterial cells (Carter et. al., 1992. Bio/Technology, 10: 163–168).

For the purposes of the present invention, the latter technique of humanizing antibodies using CDR grafting to generate humanized monoclonal antibodies or Fab derivatives thereof is the preferred technique for reducing the immunogenicity of murine monoclonal antibodies in humans.

Antibodies displayed at the surface of a bacteriophage are also contemplated by the antibodies of the invention. Bacteriophage which encode the desired antibody may be engineered such that the protein is displayed on the surface thereof in such a manner that it is available for binding to its corresponding binding protein, e.g., the antigen against which the antibody is directed. Thus, when bacteriophage which express a specific antibody are incubated in the presence of a cell which expresses the corresponding antigen, the bacteriophage will bind to the cell. Bacteriophage which do not express the antibody will not bind to the cell. Such panning techniques are well known in the art and are described for example, in Wright et al., (supra).

To generate a phage antibody library, a cDNA library is first obtained from mRNA which is isolated from cells, e.g., the hybridoma, which express the desired protein to be expressed on the phage surface, e.g., the desired antibody. cDNA copies of the mRNA are produced using reverse transcriptase. cDNA which specifies immunoglobulin fragments are obtained by PCR and the resulting DNA is cloned into a suitable bacteriophage vector to generate a bacteriophage DNA library comprising DNA specifying immunoglobulin genes. The procedures for making a bacteriophage library comprising heterologous DNA are well known in the art and are described, for example, in Sambrook et al. (1989, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor, N.Y.).

Processes such as those described above, have been developed for the production of human antibodies using M13 bacteriophage display (Burton et al., 1994, Adv. Immunol. 57:191–280). Essentially, a cDNA library is generated from mRNA obtained from a population of antibody-producing cells. The mRNA encodes rearranged immunoglobulin genes and thus, the cDNA encodes the same. Amplified cDNA is cloned into M13 expression vectors creating a library of phage which express human Fab fragments on their surface. Phage which display the antibody of interest are selected by antigen binding and are propagated in bacteria to produce soluble human Fab immunoglobulin. Thus, in contrast to conventional monoclonal antibody synthesis, this procedure immortalizes DNA encoding human immunoglobulin rather than cells which express human immunoglobulin.

The procedures just presented describe the generation of phage which encode the Fab portion of an antibody molecule. However, the invention should not be construed to be limited solely to the generation of phage encoding Fab antibodies. Rather, phage which encode single chain antibodies (scFv/phage antibody libraries) are also included in the invention. Fab molecules comprise the entire Ig light chain, that is, they comprise both the variable and constant region of the light chain, but include only the variable region and first constant region domain (CH1) of the heavy chain. Single chain antibody molecules comprise a single chain of protein comprising the Ig Fv fragment. An Ig Fv fragment includes only the variable regions of the heavy and light chains of the antibody, having no constant region contained therein. Phage libraries comprising scFv DNA may be generated following the procedures described in Marks et al., 1991, J. Mol. Biol. 222:581–597. Panning of phage so generated for the isolation of a desired antibody is conducted in a manner similar to that described for phage libraries comprising Fab DNA.

The invention should also be construed to include synthetic phage display libraries in which the heavy and light chain variable regions may be synthesized such that they include nearly all possible specificities (Barbas, 1995, Nature Medicine 1:837–839; de Kruif et al. 1995, J. Mol. Biol.248:97–105).

In one embodiment, the monoclonal antibody of the invention comprises a heavy chain polypeptide having an amino acid sequence which shares at least about 80% homology with SEQ ID NO:1 (FIG. 7A). Preferably, the heavy chain polypeptide is about 85% homologous, more preferably about 90% homologous, even more preferably about 95% homologous, and most preferably about 99% homologous to the heavy chain polypeptide of SEQ ID NO:1. Even more preferably, the monoclonal antibody of the invention comprises a heavy chain polypeptide which is SEQ ID NO:1. Also, in this embodiment the monoclonal antibody of the invention comprises a light chain polypeptide having an amino acid sequence which shares at least about 80% homology with SEQ ID NO:2 (FIG. 7B). Preferably, the light chain polypeptide is about 85% homologous, more preferably about 90% homologous, even more preferably about 95% homologous, and most preferably about 99% homologous to the light chain polypeptide of SEQ ID NO:2. Even more preferably, the monoclonal antibody of the invention comprises a light chain polypeptide which is SEQ ID NO:2.

The determination of percent homology (i.e. percent identity) described herein between two amino acid or nucleotide sequences can be accomplished using a mathematical algorithm. For example, a mathematical algorithm useful for comparing two sequences is the algorithm of Karlin and Altschul (1990, Proc. Natl. Acad. Sci. USA 87:2264–2268), modified as in Karlin and Altschul (1993, Proc. Natl. Acad. Sci. USA 90:5873–5877). This algorithm is incorporated into the NBLAST and XBLAST programs of Altschul, et al. (1990, J. Mol. Biol. 215:403-410), and can be accessed, for example, at the National Center for Biotechnology Information (NCBI) world wide web site having the universal

demulcents, preservatives, buffers, salts, flavorings, coloring agents, and sweetening agents. Oily suspensions may further comprise a thickening agent. Known suspending agents include, but are not limited to, sorbitol syrup, hydrogenated edible fats, sodium alginate, polyvinylpyrrolidone, gum tragacanth, gum acacia, and cellulose derivatives such as sodium carboxymethylcellulose, methylcellulose, hydroxypropylmethylcellulose. Known dispersing or wetting agents include, but are not limited to, naturally-occurring phosphatides such as lecithin, condensation products of an alkylene oxide with a fatty acid, with a long chain aliphatic alcohol, with a partial ester derived from a fatty acid and a hexitol, or with a partial ester derived from a fatty acid and a hexitol anhydride (e.g. polyoxyethylene stearate, heptadecaethyleneoxycetanol, polyoxyethylene sorbitol monooleate, and polyoxyethylene sorbitan monooleate, respectively). Known emulsifying agents include, but are not limited to, lecithin and acacia. Known preservatives include, but are not limited to, methyl, ethyl, or n-propyl-para- hydroxybenzoates, ascorbic acid, and sorbic acid. Known sweetening agents include, for example, glycerol, propylene glycol, sorbitol, sucrose, and saccharin. Known thickening agents for oily suspensions include, for example, beeswax, hard paraffin, and ceryl alcohol.

Liquid solutions of the active ingredient in aqueous or oily solvents may be prepared in substantially the same manner as liquid suspensions, the primary difference being that the active ingredient is dissolved, rather than suspended in the solvent. Liquid solutions of the pharmaceutical composition of the invention may comprise each of the components described with regard to liquid suspensions, it being understood that suspending agents will not necessarily aid dissolution of the active ingredient in the solvent. Aqueous solvents include, for example, water and isotonic saline. Oily solvents include, for example, almond oil, oily esters, ethyl alcohol, vegetable oils such as arachis, olive, sesame, or coconut oil, fractionated vegetable oils, and mineral oils such as liquid paraffin.

Powdered and granular formulations of a pharmaceutical preparation of the invention may be prepared using known methods. Such formulations may be administered directly to a subject, used, for example, to form tablets, to fill capsules, or to prepare an aqueous or oily suspension or solution by addition of an aqueous or oily vehicle thereto. Each of these formulations may further comprise one or more of dispersing or wetting agent, a suspending agent, and a preservative. Additional excipients, such as fillers and sweetening, flavoring, or coloring agents, may also be included in these formulations.

A pharmaceutical composition of the invention may also be prepared, packaged, or sold in the form of oil-in-water emulsion or a water-in-oil emulsion. The oily phase may be a vegetable oil such as olive or arachis oil, a mineral oil such as liquid paraffin, or a combination of these. Such compositions may further comprise one or more emulsifying agents such as naturally occurring gums such as gum acacia or gum tragacanth, naturally-occurring phosphatides such as soybean or lecithin phosphatide, esters or partial esters derived from combinations of fatty acids and hexitol anhydrides such as sorbitan monooleate, and condensation products of such partial esters with ethylene oxide such as polyoxyethylene sorbitan monooleate. These emulsions may also contain additional ingredients including, for example, sweetening or flavoring agents.

As used herein, "parenteral administration" of a pharmaceutical composition includes any route of administration characterized by physical breaching of a tissue of a subject and administration of the pharmaceutical composition through the breach in the tissue. Parenteral administration thus includes, but is not limited to, administration of a pharmaceutical composition by injection of the composition, by application of the composition through a surgical incision, by application of the composition through a tissue-penetrating non-surgical wound, and the like. In particular, parenteral administration is contemplated to include, but is not limited to, subcutaneous, intraperitoneal, intramuscular, intrasternal injection, and kidney dialytic infusion techniques. intrasternal

Formulations of a pharmaceutical composition suitable for parenteral administration comprise the active ingredient combined with a pharmaceutically acceptable carrier, such as sterile water or sterile isotonic saline. Such formulations may be prepared, packaged, or sold in a form suitable for bolus administration or for continuous administration. Injectable formulations may be prepared, packaged, or sold in unit dosage form, such as in ampules or in multi-dose containers containing a preservative. Formulations for parenteral administration include, but are not limited to, suspensions, solutions, emulsions in oily or aqueous vehicles, pastes, and implantable sustained-release or biodegradable formulations. Such formulations may further comprise one or more additional ingredients including, but not limited to, suspending, stabilizing, or dispersing agents. In one embodiment of a formulation for parenteral administration, the active ingredient is provided in dry (i.e. powder or granular) form for reconstitution with a suitable vehicle (e.g. sterile pyrogen-free water) prior to parenteral administration of the reconstituted composition.

The pharmaceutical compositions may be prepared, packaged, or sold in the form of a sterile injectable aqueous or oily suspension or solution. This suspension or solution may be formulated according to the known art, and may comprise, in addition to the active ingredient, additional ingredients such as the dispersing agents, wetting agents, or suspending agents described herein. Such sterile injectable formulations may be prepared using a non-toxic parenterally-acceptable diluent or solvent, such as water or 1,3-butane diol, for example. Other acceptable diluents and solvents include, but are not limited to, Ringer's solution, isotonic sodium chloride solution, and fixed oils such as synthetic mono- or di-glycerides. Other parentally-administrable formulations which are useful include those which comprise the active ingredient in microcrystalline form, in a liposomal preparation, or as a component of a biodegradable polymer systems. Compositions for sustained release or implantation may comprise pharmaceutically acceptable polymeric or hydrophobic materials such as an emulsion, an ion exchange resin, a sparingly soluble polymer, or a sparingly soluble salt.

Formulations suitable for topical administration include, but are not limited to, liquid or semi-liquid preparations such as liniments, lotions, oil-in-water or water-in-oil emulsions such as creams, ointments or pastes, and solutions or suspensions. Topically-administrable formulations may, for example, comprise from about 1% to about 10% (w/w) active ingredient, although the concentration of the active ingredient may be as high as the solubility limit of the active ingredient in the solvent. Formulations for topical administration may further comprise one or more of the additional ingredients described herein.

A pharmaceutical composition of the invention may be prepared, packaged, or sold in a formulation suitable for buccal administration. Such formulations may, for example, be in the form of tablets or lozenges made using conven-

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(12) **United States Patent**
Arepally et al.

(10) **Patent No.: US 6,964,854 B1**
(45) **Date of Patent: Nov. 15, 2005**

(54) **COMPOSITIONS AND METHODS USEFUL FOR THE DIAGNOSIS AND TREATMENT OF HEPARIN INDUCED THROMBOCYTOPENIA/THROMBOSIS**

(75) Inventors: **Gowthami M. Arepally**, Albuquerque, NM (US); **Walter Kisiel**, Albuquerque, NM (US); **Keiko Kamei**, Kumamoto (JP); **Shintaro Kamei**, Kumamoto (JP)

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(73) Assignee: ~~Science & Technology Corporation~~, ~~Albuquerque~~, NM (US)
Albuquerque

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 51 days.

(21) Appl. No.: **09/615,872**

(22) Filed: **Jul. 13, 2000**

Related U.S. Application Data

(60) Provisional application No. 60/143,536, filed on Jul. 13, 1999.

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(52) **U.S. Cl.** 435/7.24; 435/70.21; 435/452; 435/332; 435/337; 436/506; 530/388.2; 530/388.25

(58) **Field of Search** 435/7.24, 7.6, 435/7.92, 69.1, 70.21, 452, 332, 337; 436/506; 530/387.3, 388.1, 388.2, 388.25

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(Continued)

Primary Examiner—Christopher L. Chin
Assistant Examiner—James L. Grun
(74) *Attorney, Agent, or Firm*—Mueting, Raasch & Gebhardt, P.A.

(57) **ABSTRACT**

The invention includes compositions, kits and methods comprising a monoclonal antibody which shares key functional properties with the polyclonal antibodies which participate in the pathogenesis of heparin induced thrombocytopenia/thrombosis (HIT/HITT) in a mammal. The monoclonal antibody of the invention preferentially binds with a PF4/heparin complex relative to the binding of the antibody with PF4 or heparin alone. The monoclonal antibody of the invention also binds specifically with PF4 in a complex with other glycosaminoglycans besides heparin, and also activates platelets. The monoclonal antibody of the invention is useful in methods for diagnosing and treating HIT/HITT in a mammal. A humanized version of the monoclonal antibody of the invention is also included, along with a process for humanizing the monoclonal antibody of the invention.

6 Claims, 10 Drawing Sheets

murine antibody onto the human heavy and light chain framework residues. The original technique described by Reichman et. al. involves producing a human variable region with the desired specificity of the murine hypervariable region. Murine hypervariable (HV) primers with sequences complementary to the murine HV region are synthesized with flanking human framework sequences corresponding to the human HV region. These murine HV primers, in addition to nucleotides and DNA polymerase, are added to a plasmid containing the human variable region gene. This reaction results in a humanized murine variable gene (huV), containing human framework regions and murine HV regions. The humanized variable region is next inserted into a plasmid containing a human constant region gene.

This procedure was employed to create humanized heavy and light chains which were then cotransfected into a non-secreting myeloma cell line. Subsequent refinements of this basic procedure have resulted in a process for producing humanized antibodies with affinities and biological properties comparable to that of the parent murine monoclonal antibody (Emery et al., 1995, Strategies for humanizing antibodies, In: Antibody Engineering, 2nd ed., Ed. Borrebaeck CAK) pp 159–183, Oxford University Press, Oxford). This technology can be similary utilized to create functional humanized Fab' fragments in bacterial cells (Carter et. al., 1992. Bio/Technology, 10: 163–168).

For the purposes of the present invention, the latter technique of humanizing antibodies using CDR grafting to generate humanized monoclonal antibodies or Fab derivatives thereof is the preferred technique for reducing the immunogenicity of murine monoclonal antibodies in humans.

Antibodies displayed at the surface of a bacteriophage are also contemplated by the antibodies of the invention. Bacteriophage which encode the desired antibody may be engineered such that the protein is displayed on the surface thereof in such a manner that it is available for binding to its corresponding binding protein, e.g., the antigen against which the antibody is directed. Thus, when bacteriophage which express a specific antibody are incubated in the presence of a cell which expresses the corresponding antigen, the bacteriophage will bind to the cell. Bacteriophage which do not express the antibody will not bind to the cell. Such panning techniques are well known in the art and are described for example, in Wright et al., (supra).

To generate a phage antibody library, a cDNA library is first obtained from mRNA which is isolated from cells, e.g., the hybridoma, which express the desired protein to be expressed on the phage surface, e.g., the desired antibody. cDNA copies of the mRNA are produced using reverse transcriptase. cDNA which specifies immunoglobulin fragments are obtained by PCR and the resulting DNA is cloned into a suitable bacteriophage vector to generate a bacteriophage DNA library comprising DNA specifying immunoglobulin genes. The procedures for making a bacteriophage library comprising heterologous DNA are well known in the art and are described, for example, in Sambrook et al. (1989, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor, N.Y.).

Processes such as those described above, have been developed for the production of human antibodies using M13 bacteriophage display (Burton et al., 1994, Adv. Immunol. 57:191–280). Essentially, a cDNA library is generated from mRNA obtained from a population of antibody-producing cells. The mRNA encodes rearranged immunoglobulin genes and thus, the cDNA encodes the same. Amplified cDNA is cloned into M13 expression vectors creating a library of phage which express human Fab fragments on their surface. Phage which display the antibody of interest are selected by antigen binding and are propagated in bacteria to produce soluble human Fab immunoglobulin. Thus, in contrast to conventional monoclonal antibody synthesis, this procedure immortalizes DNA encoding human immunoglobulin rather than cells which express human immunoglobulin.

The procedures just presented describe the generation of phage which encode the Fab portion of an antibody molecule. However, the invention should not be construed to be limited solely to the generation of phage encoding Fab antibodies. Rather, phage which encode single chain antibodies (scFv/phage antibody libraries) are also included in the invention. Fab molecules comprise the entire Ig light chain, that is, they comprise both the variable and constant region of the light chain, but include only the variable region and first constant region domain (CH1) of the heavy chain. Single chain antibody molecules comprise a single chain of protein comprising the Ig Fv fragment. An Ig Fv fragment includes only the variable regions of the heavy and light chains of the antibody, having no constant region contained therein. Phage libraries comprising scFv DNA may be generated following the procedures described in Marks et al., 1991, J. Mol. Biol. 222:581–597. Panning of phage so generated for the isolation of a desired antibody is conducted in a manner similar to that described for phage libraries comprising Fab DNA.

The invention should also be construed to include synthetic phage display libraries in which the heavy and light chain variable regions may be synthesized such that they include nearly all possible specificities (Barbas, 1995, Nature Medicine 1:837–839; de Kruif et al. 1995, J. Mol. Biol.248:97–105).

In one embodiment, the monoclonal antibody of the invention comprises a heavy chain polypeptide having an amino acid sequence which shares at least about 80% homology with SEQ ID NO:1 (FIG. 7A). Preferably, the heavy chain polypeptide is about 85% homologous, more preferably about 90% homologous, even more preferably about 95% homologous, and most preferably about 99% homologous to the heavy chain polypeptide of SEQ ID NO:1. Even more preferably, the monoclonal antibody of the invention comprises a heavy chain polypeptide which is SEQ ID NO:1. Also, in this embodiment the monoclonal antibody of the invention comprises a light chain polypeptide having an amino acid sequence which shares at least about 80% homology with SEQ ID NO:2 (FIG. 7B). Preferably, the light chain polypeptide is about 85% homologous, more preferably about 90% homologous, even more preferably about 95% homologous, and most preferably about 99% homologous to the light chain polypeptide of SEQ ID NO:2. Even more preferably, the monoclonal antibody of the invention comprises a light chain polypeptide which is SEQ ID NO:2.

The determination of percent homology (i.e. percent identity) described herein between two amino acid or nucleotide sequences can be accomplished using a mathematical algorithm. For example, a mathematical algorithm useful for comparing two sequences is the algorithm of Karlin and Altschul (1990, Proc. Natl. Acad. Sci. USA 87:2264–2268), modified as in Karlin and Altschul (1993, Proc. Natl. Acad. Sci. USA 90:5873–5877). This algorithm is incorporated into the NBLAST and XBLAST programs of Altschul, et al. (1990, J. Mol. Biol. 215:403-410), and can be accessed, for example, at the National Center for Biotechnology Information (NCBI) world wide web site having the universal

215:403-410

demulcents, preservatives, buffers, salts, flavorings, coloring agents, and sweetening agents. Oily suspensions may further comprise a thickening agent. Known suspending agents include, but are not limited to, sorbitol syrup, hydrogenated edible fats, sodium alginate, polyvinylpyrrolidone, gum tragacanth, gum acacia, and cellulose derivatives such as sodium carboxymethylcellulose, methylcellulose, hydroxypropylmethylcellulose. Known dispersing or wetting agents include, but are not limited to, naturally-occurring phosphatides such as lecithin, condensation products of an alkylene oxide with a fatty acid, with a long chain aliphatic alcohol, with a partial ester derived from a fatty acid and a hexitol, or with a partial ester derived from a fatty acid and a hexitol anhydride (e.g. polyoxyethylene stearate, heptadecaethyleneoxycetanol, polyoxyethylene sorbitol monooleate, and polyoxyethylene sorbitan monooleate, respectively). Known emulsifying agents include, but are not limited to, lecithin and acacia. Known preservatives include, but are not limited to, methyl, ethyl, or n-propyl-para- hydroxybenzoates, ascorbic acid, and sorbic acid. Known sweetening agents include, for example, glycerol, propylene glycol, sorbitol, sucrose, and saccharin. Known thickening agents for oily suspensions include, for example, beeswax, hard paraffin, and cetyl alcohol.

Liquid solutions of the active ingredient in aqueous or oily solvents may be prepared in substantially the same manner as liquid suspensions, the primary difference being that the active ingredient is dissolved, rather than suspended in the solvent. Liquid solutions of the pharmaceutical composition of the invention may comprise each of the components described with regard to liquid suspensions, it being understood that suspending agents will not necessarily aid dissolution of the active ingredient in the solvent. Aqueous solvents include, for example, water and isotonic saline. Oily solvents include, for example, almond oil, oily esters, ethyl alcohol, vegetable oils such as arachis, olive, sesame, or coconut oil, fractionated vegetable oils, and mineral oils such as liquid paraffin.

Powdered and granular formulations of a pharmaceutical preparation of the invention may be prepared using known methods. Such formulations may be administered directly to a subject, used, for example, to form tablets, to fill capsules, or to prepare an aqueous or oily suspension or solution by addition of an aqueous or oily vehicle thereto. Each of these formulations may further comprise one or more of dispersing or wetting agent, a suspending agent, and a preservative. Additional excipients, such as fillers and sweetening, flavoring, or coloring agents, may also be included in these formulations.

A pharmaceutical composition of the invention may also be prepared, packaged, or sold in the form of oil-in-water emulsion or a water-in-oil emulsion. The oily phase may be a vegetable oil such as olive or arachis oil, a mineral oil such as liquid paraffin, or a combination of these. Such compositions may further comprise one or more emulsifying agents such as naturally occurring gums such as gum acacia or gum tragacanth, naturally-occurring phosphatides such as soybean or lecithin phosphatide, esters or partial esters derived from combinations of fatty acids and hexitol anhydrides such as sorbitan monooleate, and condensation products of such partial esters with ethylene oxide such as polyoxyethylene sorbitan monooleate. These emulsions may also contain additional ingredients including, for example, sweetening or flavoring agents.

As used herein, "parenteral administration" of a pharmaceutical composition includes any route of administration characterized by physical breaching of a tissue of a subject and administration of the pharmaceutical composition through the breach in the tissue. Parenteral administration thus includes, but is not limited to, administration of a pharmaceutical composition by injection of the composition, by application of the composition through a surgical incision, by application of the composition through a tissue-penetrating non-surgical wound, and the like. In particular, parenteral administration is contemplated to include, but is not limited to, subcutaneous, intraperitoneal, intramuscular, intrasternal injection, and kidney dialytic infusion techniques. intrasternal

Formulations of a pharmaceutical composition suitable for parenteral administration comprise the active ingredient combined with a pharmaceutically acceptable carrier, such as sterile water or sterile isotonic saline. Such formulations may be prepared, packaged, or sold in a form suitable for bolus administration or for continuous administration. Injectable formulations may be prepared, packaged, or sold in unit dosage form, such as in ampules or in multi-dose containers containing a preservative. Formulations for parenteral administration include, but are not limited to, suspensions, solutions, emulsions in oily or aqueous vehicles, pastes, and implantable sustained-release or biodegradable formulations. Such formulations may further comprise one or more additional ingredients including, but not limited to, suspending, stabilizing, or dispersing agents. In one embodiment of a formulation for parenteral administration, the active ingredient is provided in dry (i.e. powder or granular) form for reconstitution with a suitable vehicle (e.g. sterile pyrogen-free water) prior to parenteral administration of the reconstituted composition.

The pharmaceutical compositions may be prepared, packaged, or sold in the form of a sterile injectable aqueous or oily suspension or solution. This suspension or solution may be formulated according to the known art, and may comprise, in addition to the active ingredient, additional ingredients such as the dispersing agents, wetting agents, or suspending agents described herein. Such sterile injectable formulations may be prepared using a non-toxic parenterally-acceptable diluent or solvent, such as water or 1,3-butane diol, for example. Other acceptable diluents and solvents include, but are not limited to, Ringer's solution, isotonic sodium chloride solution, and fixed oils such as synthetic mono- or di-glycerides. Other parentally-administrable formulations which are useful include those which comprise the active ingredient in microcrystalline form, in a liposomal preparation, or as a component of a biodegradable polymer systems. Compositions for sustained release or implantation may comprise pharmaceutically acceptable polymeric or hydrophobic materials such as an emulsion, an ion exchange resin, a sparingly soluble polymer, or a sparingly soluble salt.

Formulations suitable for topical administration include, but are not limited to, liquid or semi-liquid preparations such as liniments, lotions, oil-in-water or water-in-oil emulsions such as creams, ointments or pastes, and solutions or suspensions. Topically-administrable formulations may, for example, comprise from about 1% to about 10% (w/w) active ingredient, although the concentration of the active ingredient may be as high as the solubility limit of the active ingredient in the solvent. Formulations for topical administration may further comprise one or more of the additional ingredients described herein.

A pharmaceutical composition of the invention may be prepared, packaged, or sold in a formulation suitable for buccal administration. Such formulations may, for example, be in the form of tablets or lozenges made using conven-